

RECTAL GLAND OF FRESHWATER STINGRAYS, *POTAMOTRYGON* SPP. (CHONDRICHTHYES: POTAMOTRYGONIDAE)

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The rectal salt gland of elasmobranchs (also known in the English literature as the caecal, cloacal, anal, superanal, rectal, vermiform and digitiform gland, process or appendage) has been amply treated in the older literature (Hoskins, 1917; Crofts, 1925). The gland has been likened to the ink sac of cephalopods, various intestinal diverticula found in other vertebrates and a urinary bladder, and has been assigned digestive, reproductive, secretory and blood-cleansing functions. More than sixty years before the discovery of its true function, Crawford (1899, p. 60) stated, "The rich blood supply, the character of the secreting cells, resembling so closely as they do the cells of the kidney, and the occurrence of urea in considerable amount in the secretion, all point to the structure having an excretory function, and playing the part of a supplementary kidney." Crawford has not been given appropriate credit for his prescience, which was essentially confirmed by Burger and Hess (1960) when they demonstrated that the rectal gland of *Squalus acanthias* secretes sodium chloride in a concentration approximately twice that of the plasma.

Perhaps because of the technical difficulties in collecting the rectal gland fluid, except for *S. acanthias*, it has been collected and analyzed only from the lip shark, *Hemiscyllium plagiosum* (Chan, Phillips and Chester Jones, 1967) and the stingray, *Dasyatis sabina* (Burger, 1972; Beitz, 1977). It can nevertheless be reasonably assumed that the salt secreting function of the rectal gland is universal among marine elasmobranchs.

Since the rectal gland functions to rid the body of excess salt, it is reasonable to expect that secretion would stop in a euryhaline species when it enters fresh water. Although this has not yet been conclusively demonstrated, Oguri (1964) and Gerzeli, Gervaso and De Stefano (1969) have noted that the rectal glands of *Carcharhinus leucas*, the highly euryhaline bull shark, taken from a freshwater environment, are smaller than in the same species taken from marine water. Furthermore, by histological examination, they noted concomitant regressive changes in the secretory tubules of the freshwater specimens.

Carcharhinus leucas moves back and forth between fresh water and the sea (Thorson, 1971) and can readily tolerate both media (Thorson and Gerst, 1972). Presumably, when movement takes place between salt and fresh water the rectal gland alternates between activity and inactivity. However, the family Potamotrygonidae, freshwater stingrays of South American river systems, live permanently in fresh water and have apparently been limited to fresh water for a very long time. They no longer retain high concentrations of urea, so uni-

¹ Deceased October 26, 1975.

versally employed as an osmoregulatory agent by marine and euryhaline elasmobranchs (Thorson, Cowan and Watson, 1967; Junqueira, Hoxter and Zago, 1968); nor do they build up their urea content when transferred to varying dilutions of sea water (Thorson, 1970; Griffith, Pang, Srivastava and Pickford, 1973; Gerst and Thorson, 1977). The urea retaining ability is apparently of no further survival value to elasmobranchs in a freshwater environment. Since salts are in extremely short supply in the fresh water of tropical South American rivers, an interesting question is posed concerning the fate of the strictly freshwater stingray's rectal gland, for whose salt-secreting function there is likewise no further use.

This paper presents findings concerning the morphological aspects of this question, as well as some of their physiological connotations.

MATERIALS AND METHODS

Freshwater stingrays of the genus *Potamotrygon* were procured at Leticia, Colombia, from the Amazon River drainage in Brazil and from aquarium suppliers in the United States. The latter specimens were imported from dealers on the Amazon River and were clearly rays of the subject genus, although identification to species was not always possible.

Sections were made of portions of appropriate tissues from numerous rays, but the illustrations and discussion are based primarily on two: specimen A, a juvenile female (*Potamotrygon motoro*) of 160 mm disc width, purchased from a Nebraska supplier; and specimen B, a female approaching sexual maturity (*P. circularis*), 413 mm disc width, taken by a local dealer from the Itacoai River, an Amazon tributary in extreme western Brazil, near Leticia, Colombia.

From the rays selected for study, a section of the lower end of the alimentary canal, with associated structures, was immersed either directly in Bouin's fluid or in 10% formalin and later transferred to Bouin's. Specimens were transferred through several changes of 70% alcohol to remove the excess Picric acid. All the tissues were passed through successive increasing strengths of alcohol up to absolute, to insure thorough dehydration. The last change of 100% alcohol was replaced with xylene to which dryrite had been added. After clearing, they were placed in molten paraffin and subsequently embedded in wax. Sections were cut at 8 to 10 micra and the ribbons affixed to slides with Meyer's albumin fluid. The sections were stained with Ehrlich's acid hematoxylin and Eosin as tinctorial agents, and mounted in balsam under glass cover slips.

Micrographs were taken with a Zeiss photo-microscope II on Ilford Pan F film.

RESULTS

The rectal gland and associated tissues of specimen A (a juvenile female *Potamotrygon motoro*) are shown in Figure 1. The gland is a short, slender structure, directed anteriorly from the dorsal side of the post-valvular intestine. It is closely associated with three ovoid masses of white tissue. Both the gland and the three white lobes are covered with peritoneum. The same structures and arrangement were found in specimen B, a female *P. circularis* approaching sexual

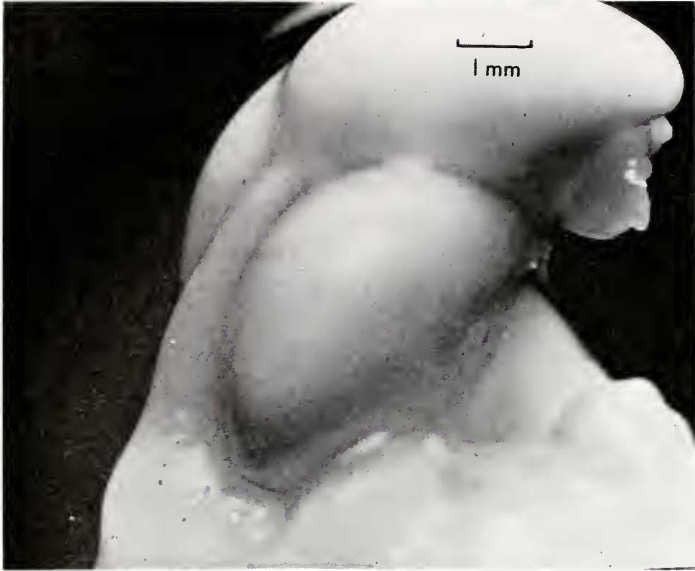


FIGURE 1. Rectal gland and associated myeloid lobes of a juvenile *Potamotrygon motoro* (160 mm disc width). The gland is the slender structure at left.

maturity (Fig. 2). In this larger animal the white masses have become further lobed and irregular in shape.

A representative cross section of the rectal gland from specimen A is shown in Figure 3 and an enlarged area of the section in Figure 4. The glandular portion, surrounding a central lumen (A), occupies approximately the central half of the gland's diameter. It includes a series of tubules (B) which are composed of simple cuboidal cells and drain into the central lumen, as at C. Surrounding the central glandular portion and forming most of the remainder of the gland, is a broad band of connective tissue containing blood vessels (D) and sinuses. The free surface of the gland is covered by a stratified columnar epithelium (E). The central lumen is lined with a simple squamous epithelium which becomes stratified as it comes closer to and enters the rectum.

The gland, although in close connection with the associated lobes (Fig. 1), is clearly and completely independent, being separated from them by a broad layer of connective tissue (Fig. 3).

Examination of the lobes associated with the rectal gland discloses an external epithelial layer continuous with that of the gland. The epithelium covers a thin connective tissue stratum and inside is a heavy concentration of leucocytes of various kinds and stages, including some with mitotic figures.

DISCUSSION

The white lobes associated with the rectal gland undoubtedly represent the "lymphoid tissue" described and figured in several earlier accounts of the gross structure of the rectal gland (*e.g.*, Hoskins, 1917). Their histology indicates

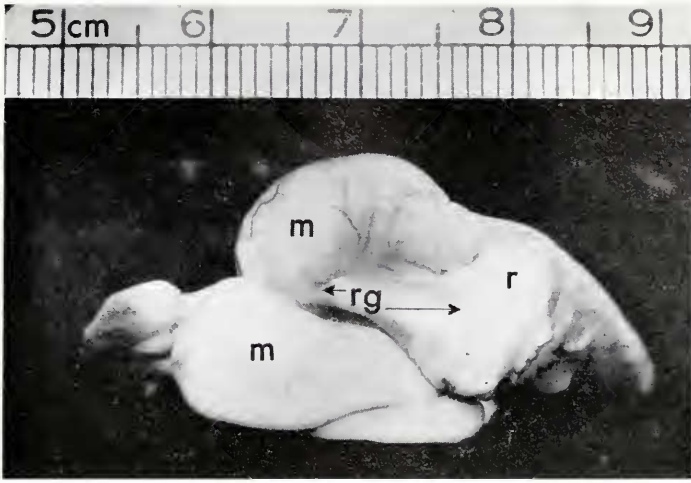


FIGURE 2. Rectal gland (rg) with portion of rectum (r) and associated myeloid lobes (m) of a female *Potamotrygon circularis* nearing sexual maturity (413 mm disc width).

that they are a part of the lymphomyeloid system of cartilaginous fishes recently discussed by Fänge (1977). This system is active in haemopoiesis and in the immune responses. Components of the system mentioned by Fänge include the spleen and thymus; the epigonal organs (associated with the gonads); Leydig's organ in the esophagus; extensive tissue in the cranium (in holocephalans); and aggregations of leucocytes in the connective tissue of the kidneys and the intestine (spiral valve).

The prominence and distinctness of the organs discussed here and their close

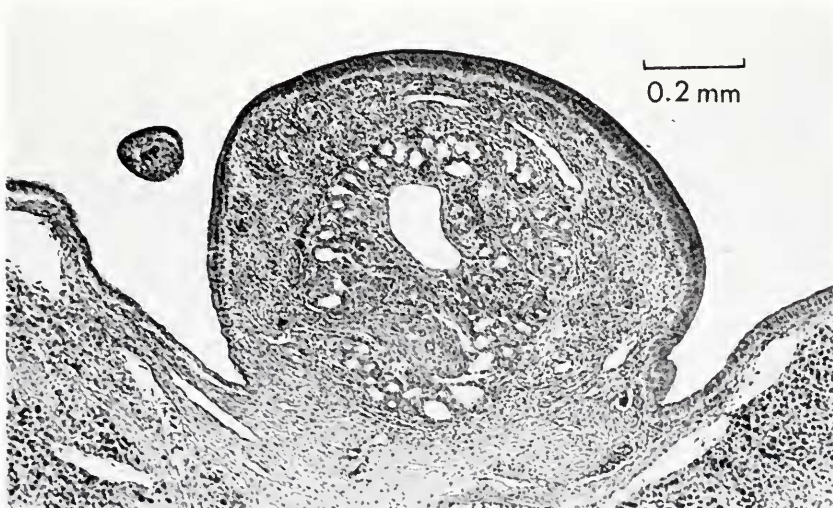


FIGURE 3. Cross section of rectal gland shown in Figure 1.

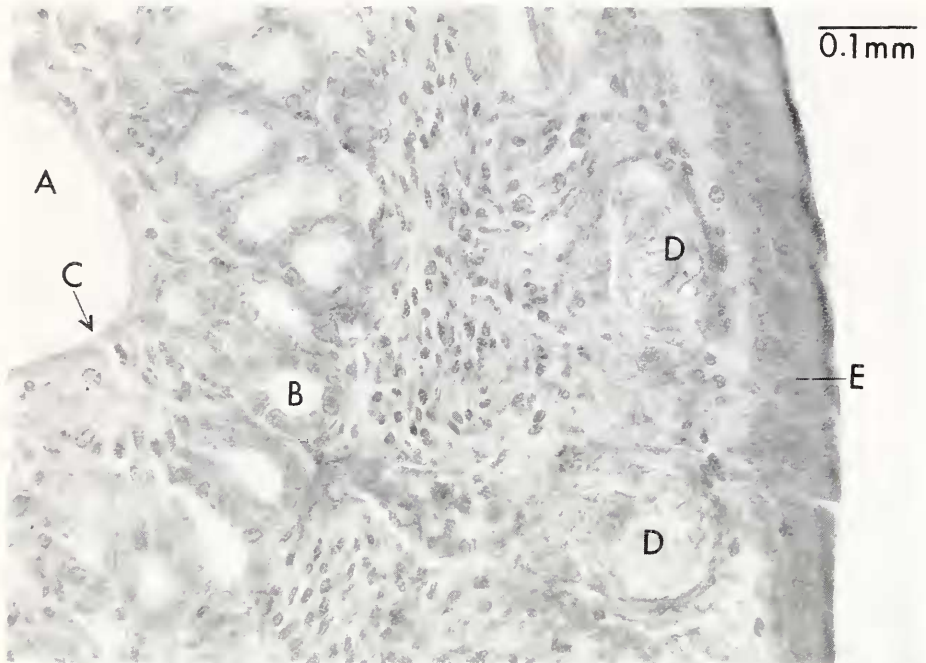


FIGURE 4. Enlarged area of rectal gland section (from Fig. 3): a central lumen (A), lined with simple, squamous epithelium, is surrounded by scattered tubules (B) which empty into lumen, as at C. A wide band of connective tissue, with blood vessels (D) occupies the outer portion of the section, and the gland is covered with a stratified, columnar epithelium (E).

association with the rectal gland and post-valvular gut justify their designation as rectomyeloid bodies.

Goldstein and Forster (1971) were unable to find a rectal gland in *Potamotrygon* sp. Griffith *et al.* (1973) reported that rays studied by them (*Potamotrygon* spp.) had an organ in the anatomical position of the rectal gland, but histological investigation showed that it was structurally unlike the rectal gland of marine elasmobranchs. Gerzeli *et al.* (1969) and Gerzeli, De Stefano, Bolognani, Koenig, Gervaso and Omodeo-Salé (1976) reported a rectal gland in a stingray identified as *Potamotrygon brachyurus* and noted that it was large, with a gland weight/body weight ratio of *ca.* 1×10^{-3} . Gerst and Thorson (1977) reported the presence of a structure in *Potamotrygon* spp. with the location and histological characteristics of the elasmobranch rectal gland, but of reduced proportions.

The present study conclusively establishes the presence of a rectal gland in the Potamotrygonidae, with the location and histological features of the gland found in marine elasmobranchs. The confusion and conflicting reports cited above may be explained by the fact that the gland is small and inconspicuous and may easily be obscured by, or even mistaken for, a part of the lobed myeloid tissue spatially associated with it.

Marine and brackishwater elasmobranchs have relatively large glands (Table I). The fully euryhaline shark (*Carcharhinus leucas*) that completely tolerates both

TABLE I

Rectal gland/body weight ratio related to habitat.

Reference	Species	Rectal gland wt./body wt. ratio (units per million)
<i>Marine and brackish water species</i>		
Burger (1972)	<i>Squalus acanthias</i>	600
	<i>Dasyatis sabina</i>	240
Fänge and Fugelli (1963)	<i>Selache maxima</i>	200
Chan and Phillips (1967)	<i>Hemiscyllium plagiosum</i>	120
Bonting (1966)	<i>Squalus acanthias</i>	444
	<i>Carcharius littoralis</i>	185
	<i>Carcharhinus falciformis</i>	190
	<i>Mustelus canis</i>	214
	<i>Raja eglanteria</i>	202
	<i>Myliobatus freminvillei</i>	164
	<i>Pteroplatea altavela</i>	86
	<i>Squatina squatina</i>	99
<i>Fully euryhaline species</i>		
Gerzeli <i>et al.</i> (1969)	<i>Carcharhinus leucas</i> (marine)	60
	<i>Carcharhinus leucas</i> (fresh water)	20
Thorson (unpublished)	<i>Carcharhinus leucas</i> (fresh water)	30
<i>Freshwater species</i>		
Thorson (unpublished)	<i>Potamotrygon circularis</i>	15
Gerzeli <i>et al.</i> (1969)	<i>Potamotrygon brachyurus</i>	1000*

* See text.

fresh and salt water has a gland of somewhat reduced relative size; the gland appears to be larger when this shark is in sea water than when it is in fresh water (Oguri, 1964; Gerzeli *et al.*, 1969). The completely freshwater rays (Potamotrygonidae) examined in this study have rectal glands of still more reduced size. Furthermore, the number of tubules is considerably reduced and their distribution within the gland is relatively restricted (Fig. 3). The rectal gland weight/body weight ratio of 1×10^{-3} given by Gerzeli *et al.* (1969) is greater than that of any marine elasmobranch listed in Table I. The figure must either be in error or the specimen studied may have included myeloid or other tissue in addition to the gland itself.

Atrophy of the gland might reasonably be expected in rays that have been completely limited to fresh water for a long, although undetermined, period of time. Just as they have abandoned urea retention, the freshwater rays apparently have also abandoned supplementary salt excretion. Both would be counter-productive in a freshwater environment.

The highly euryhaline *Carcharhinus leucas* is able to increase and decrease the urea content of its body fluids in response to changes in environmental salinity. The findings of Oguri (1964), and Gerzeli *et al.* (1969; 1976) suggest that secretory activity of the rectal gland of *C. leucas* also responds to changes in environ-

mental salinity. In *Potamotrygon* spp., on the other hand, transfer to saline environment does not elicit an increase in urea concentration in body fluids, and the loss of urea retention appears to be irreversible (Thorson, 1970; Griffith *et al.*, 1973; Gerst and Thorson, 1977). This fact suggests that the apparent loss of salt secretory activity of the atrophied rectal gland in *Potamotrygon* may also be irreversible. This view is supported by the observation that, in potamotrygonids transferred to dilute sea water, regulation of inorganic ions breaks down. Sodium and chloride concentrations in particular almost double in seawater-acclimated rays. The greatest concentration they can tolerate for any length of time is approximately 40% sea water (Thorson *et al.*, 1967; Thorson, 1970; Griffith *et al.*, 1973; Gerst and Thorson, 1977).

No function, other than salt secretion, has been demonstrated for the fully active rectal gland of marine elasmobranchs. What residual function the atrophied potamotrygonid rectal gland might have, if any, is unknown. Gerzeli *et al.*, (1976, p. 619) reported that the rectal gland of *Potamotrygon brachyurus* "appears very peculiar, showing secretory activity histologically, but lacking any cytochemical evidence related to salt secretion." Otherwise, nothing has been established experimentally or histochemically concerning any specific function for the potamotrygonid rectal gland.

The Chondrichthyes made their appearance in the geological record during the Devonian. Although their presumed ancestors, the placoderms, may have inhabited inland fresh waters, the Devonian Chondrichthyes appear to have been marine since their first appearance (Romer, 1966). This study does little to elucidate the continuing discussion of whether urea retention developed in chondrichthians in response to the invasion of salt water or existed earlier and provided a pre-adaptation for marine life. However, it provides evidence bearing on a related question concerning the potamotrygonid stingrays: does the near absence of urea in the freshwater rays represent a genetic deletion of their ancestral ability to concentrate urea, or were they descended from ancestors that had never left fresh water and had never developed urea retention? The latter possibility was considered remote by Thorson *et al.* (1967) and less plausible than the former by Forster and Goldstein (1969). However, evidence concerning urea retention is not preserved in fossils, and there is in any case no fossil record of the family Potamotrygonidae. Stingrays of the closely related marine family Dasyatidae are known from freshwater assemblages of the Tertiary (Eocene), but reports of fossil Potamotrygonidae in South America (Garman, 1913) and in Africa (Arambourg, 1947) are probably also of dasyatids (Thorson and Watson, 1975).

In the absence of fossil evidence of the history of this group, evidence must be sought from extant rays. Such evidence is now provided by the rectal gland, which is likewise not preserved in fossils. A functional rectal gland can only be viewed as a marine adaptation and its presence, albeit in much reduced form, with no known function, can only indicate a marine ancestry for the freshwater stingrays. The chronology of the gland's earliest history cannot at present be firmly established, but at the time of the first appearance of the stingrays, in the Cretaceous (Romer, 1966), they were almost certainly already marine, as were the other Chondrichthyes, and possessed the functional rectal gland so universally found in the other cartilaginous fishes.

Both the absence of urea retention and the atrophy of the rectal gland bespeak a long history in fresh water for the Potamotrygonidae. The salinity tolerance, urea retaining ability and the size and condition of the rectal gland, studied in a variety of stingray species representing the full spectrum of environmental salinities, are potentially rich sources of evidence regarding the evolution of freshwater adaptation in stingrays as well as elasmobranchs in general.

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SUMMARY

1. Contrary to some reports, a rectal gland is present in strictly freshwater stingrays of South American rivers (*Potamotrygon* spp.).
2. The gland has the location and histological features of the salt-secreting rectal gland of marine elasmobranchs, but is much reduced in size and number of tubules.
3. Its residual function, if any, is unknown.
4. The rectal gland is associated with prominent myeloid lobes, here designated as rectomyeloid bodies.
5. In the absence of potamotrygonid fossils, the atrophied rectal gland is strong evidence of marine ancestry for the freshwater rays.
6. Both the reduced gland and the loss of urea retention in potamotrygonids are indicative of a long history of freshwater adaptation.

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